

A CYTOLOGICAL AND MORPHOLOGICAL STUDY OF CULTIVATED CYNODON SPECIES.

(With Plate XVII).

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INTRODUCTION :

Many problems have arisen in connection with the systematics of the genus *Cynodon* and there is great necessity that these anomalies be cleared up and the genus thoroughly revised. The confused taxonomy is due to the wide range of variability existing within so-called species and also to the large number of intergrading forms that occur between these species.

The study of this genus was undertaken with the object of disclosing definite characteristics which would enable workers to distinguish between species and varieties of *Cynodon* and to identify isolated plants.

As the increasing interest in turf problems which results from the growing demand for ornamental lawns, sports fields and aerodromes has emphasized the value of *Cynodon* species as lawn grasses, it was decided to commence the work with the study of grasses in common use. The five lawn grasses selected are known to gardeners and field workers as "Coarse kweek" (*Cynodon dactylon* Pers.), "Florida" (*Cynodon transvaalensis* Burt Davy), "Bradley" (*Cynodon Bradleyi* Stent), "Magennis" and "Hall's Selection". The three first mentioned are recognised species. The writer found "Magennis" to be a hybrid between *Cynodon dactylon* and *Cynodon transvaalensis*. Because this hybrid reproduces vegetatively and so remains true to type, it has been given specific rank and is referred to as *Cynodon Magennisii* sp. nov. "Hall's Selection" was found to be a variety of *Cynodon dactylon*; it is referred to as *Cynodon dactylon* Pers. var. *densus* var. nov. owing to its compact habit of growth.

Before the commencement of this work the five grasses selected had been growing in the experimental plots at Frankenwald (the Botanical Research Station of the University of the Witwatersrand) for at least three years, and had throughout this period retained their original distinguishing features.

It was decided to base the classification of the *Cynodon* genus on a study of external morphology, leaf anatomy and chromosomes.

EXTERNAL MORPHOLOGY :

After a critical and detailed examination of the external morphology of the *Cynodon* species studied, it was concluded that these species do not differ greatly, and that the only reliable and constant characters are the vernation of the leaves, the presence or absence of rhizomes, the nature of the produced rachilla, the number of primary nerves, and the length of the glumes in relation to that of the spikelet. In addition, the length, width and hairiness of the leaf, the number of spikes, the nature of the ligule, and size, are characters which are useful diagnostically when they are considered not individually but in conjunction with other characters.

Cynodon Bradleyi is a distinct species which differs from the other *Cynodon* species studied in the following respects :

- (a) no underground rhizomes are present.
- (b) the leaves are rolled in the bud.
- (c) the leaves are very hairy on both surfaces, with hairs up to 1.5 mm. long. (The hairiness of the leaves appears to be a constant character in this species).
- (d) the ligule is usually distinctly membranous and up to 1 mm. long.
- (e) the rachilla when produced is a short bristle less than 1 mm. long, which never bears a reduced floret.

Cynodon transvaalensis differs from the other *Cynodon* species studied in that there are only 3 primary nerves in the leaf blade. In addition, this species is characterised by leaves less than 1.5 mm. wide, 1 to 3 (usually 2) spikes, glumes $\frac{1}{4}$ to $\frac{1}{3}$ the length of the spikelet, and a produced rachilla which never bears a reduced floret.

Cynodon dactylon is distinctive in that the glumes may be almost as long as the spikelet. This grass is more vigorous and has a more robust habit than any of the other forms ; the spikes may be as many as 6 and 1.5 to 6.5 cm. long, the leaves are 3 to 4 mm. wide and 2 to 16 cm. long, and the flowering culms may grow to a height of 40 cm.

Cynodon dactylon var. *densus* (Hall's Selection) is closely similar to *Cynodon dactylon* in all essential features of external morphology. Since these two grasses differ only in size and habit, it would appear that "Hall's Selection" is merely a prostrate, slow-growing variety of *Cynodon dactylon*.

***Cynodon dactylon* Pers. var. *densus* var. nov.**

A *C. dactylon* differt habitu repentiore, densiore caespite et auctu tardiore.

C. Magennisii closely resembles both *Cynodon dactylon* and *Cynodon transvaalensis* in external morphology, and appears to be intermediate

between these two species. In general appearance *Cynodon Magennisii* is very similar to *Cynodon transvaalensis*; the two grasses are both fine lawn grasses, with the same growth form, and fairly short narrow leaves sparsely hairy on both surfaces. However, as regards the number of primary nerves in the leaf blade, and the presence of a reduced floret borne on the produced rachilla, *Cynodon Magennisii* is closely related to *Cynodon dactylon*.

***Cynodon Magennisii*, sp. nov.**

C. transvaalensis × *C. dactylon*; *C. transvaalensem* magnitudine et habitu revocat.

Perennis; repens, dense caespitosa, stolonifera, et rhizomis subterraneis; *culmi* tenues, erecti, 3—6 cm. alti; *folia* ab initio plicata deinde angusta linearia, fusca viridia nec glauca; *vagina* 0.4—1.5 cm. longa; *lamina* 0.5—4.5 cm. longa, basim 2 mm. lata, nervibus praecipuis 5, supra subtusque papillis et ciliis sparsis 0.8 mm. longis; *ligula* 0.2 mm. longa, membranacea vel ad seriem ciliatam reducta; *spicae* 3 vel 4, digitatae, 1.5—2.5 cm. longae; *rachilla* prolongata, tenuis, 1 mm. longa, saepe flosculo reducto; *spicula* sterilis, ovata, 2 mm. longa; *glumae* subaequales et acutae, dimidia spiculae parte longae, uninerves; *lemma* trinervis, nec carinata, apice subacuto, 2 mm. longa; *palea* 2 mm. longa.

LEAF ANATOMY:

The anatomical structure of the leaves of the *Cynodon* species studied was found to be closely similar. The only reliable diagnostic features are the nature of the abaxial epidermis (the presence or absence of tubercle-based hairs and papillae, and the thickness of the outer cell wall), and the number of first order bundles present.

Fisher (1940) uses as diagnostic characters not only the nature of the abaxial epidermis and the number of first order bundles, but also the occurrence of parenchyma in the midrib. According to Stent (1927) the presence of parenchyma between the bundles is an important specific character. The writer considers the occurrence of parenchyma, both in the midrib and between the bundles, to be variable and unreliable diagnostically.

Cynodon transvaalensis is characterised by the presence of only three first order bundles, and *Cynodon dactylon* by the unusual nature of the abaxial epidermis. A comparison of the leaf anatomy appears to support the contention that *Cynodon Magennisii* is a hybrid—the result of a cross between *Cynodon dactylon* and *Cynodon transvaalensis*. On the

whole, the internal structure of the leaf of *Cynodon Magennisii* more closely resembles that of *Cynodon transvaalensis*, particularly as regards the nature of the abaxial epidermis; however, the presence of five first order bundles establishes its affinity with *Cynodon dactylon*. The leaf anatomy of *Cynodon dactylon* var. *densus* was, for all practical purposes, identical with that of *Cynodon dactylon*, although these two grasses are very different in habit and growth form. It is of interest that the leaf structure of *Cynodon Bradleyi* is so similar to that of the other *Cynodon* species studied, considering that, with respect to the rolling of the leaves in the bud, it is so distinct. The only anatomical feature which separates it from the other species is the excessive hairiness of the leaf.

These anatomical features have been used in conjunction with the external morphological characters, to produce the following key for the classification of the different species:

ARTIFICIAL KEY TO THE SPECIES.

- | | |
|--|--|
| 1. Leaves folded in the bud | 2 |
| Leaves rolled in the bud; leaves densely hairy on both surfaces with hairs 1.5 mm. long; rachilla sometimes produced but never bearing a reduced floret; ligule usually distinctly membranous; underground rhizomes absent | <i>Cynodon Bradleyi</i> . |
| 2. 5 primary nerves in the leaf-blade | 3 |
| 3 primary nerves in the leaf-blade; 1 to 3 (usually 2) spikes; rachilla always produced but never bearing a reduced floret; glumes $\frac{1}{2}$ to $\frac{1}{3}$ the length of the spikelet; leaves not more than 1.5 mm. wide; a fine lawn grass | <i>Cynodon transvaalensis</i> . |
| 3. Both surfaces of the leaf conspicuously papillate, and sparsely covered with hairs; glumes $\frac{1}{2}$ length of spikelet; leaves less than 2 mm. wide; a fine lawn grass | <i>Cynodon Magennisii</i> . |
| Abaxial surface of the leaf without distinct papillae and usually glabrous; glumes more than $\frac{1}{2}$ the length of the spikelet; leaves not more than 2 mm. wide; coarse grasses | 4 |
| 4. Spikes 2 to 6, 1.5 to 6.5 cm. long; leaves 2 to 16 cm. long and 3 to 4 mm. wide; flowering culm up to 40 cm. high; hairs on leaf up to 1.5 mm. long; a vigorous grass | <i>Cynodon dactylon</i> . |
| Spikes 2 to 4, 1.5 to 2.5 cm. long; leaves 0.5 to 3 cm. long and 2 to 3 mm. wide; flowering culm up to 10 cm. high; hairs on leaf not more than 0.5 mm. long; a slow-growing prostrate grass | <i>Cynodon dactylon</i> var. <i>densus</i> . |

CHROMOSOMES:

Previous work on Cynodon Chromosomes:

Avdulow (1931) records a diploid chromosome number of 36 for *Cynodon dactylon* Pers., and maintains that the basic number of the genus is 9.

Hunter (1934), on the other hand, found a somatic chromosome number of 30 in *Cynodon dactylon* Pers., and states that the basic number appears to be 10.

Darlington and Janaki Ammal (1945) consider 9 to be the basic number of the *Cynodon* genus. They give the following chromosome counts :—

<i>Cynodon diploidum</i> n. sp. (Giant Star Grass)—18—	Janaki Ammal, unpublished count.
<i>Cynodon dactylon</i> (Bermuda Grass)	—36—Avdulow, 1931.
	—36—Janaki Ammal, unpublished count.
	—30 ?—Hunter, 1934.

The chromosome numbers given are the somatic or diploid numbers. The question mark is added after Hunter's number, as the writers (Darlington and Janaki Ammal) are uncertain of this number or of its attachment to the botanical name given.

METHODS :

Root tips were fixed in Navashin's fluid, sectioned transversely at 7 microns, and stained with crystal violet.

Critical observations of chromosomes were made with a Bausch and Lomb 2 mm. (90 ×) apochromatic oil immersion objective and a Zeiss 17 × ocular, giving a total magnification of 3,000 × at table level. The diagrams of the metaphase plates were drawn at this magnification with the aid of a Zeiss camera lucida. Photographs of the same metaphase plates were taken at a magnification of 1,150 × .

RESULTS :

The following chromosome counts were made from metaphase plates in root tip cells :

<i>Cynodon Bradleyi</i> Stent	18
<i>Cynodon transvaalensis</i> Burt Davy	20
<i>Cynodon dactylon</i> Pers.	40
<i>Cynodon dactylon</i> Pers. var. <i>densus</i> var. nov.	40
<i>Cynodon Magennisii</i> sp. nov.	30

DISCUSSION OF RESULTS :

It is obvious from an examination of the somatic chromosomes of the *Cynodon* species studied that the chromosomes in each complement, because they are small and remarkably uniform, cannot be distinguished morphologically. Any cytological classification must therefore be based entirely on differences in chromosome number.

With the exception of *Cynodon Bradleyi*, the somatic chromosome numbers of the *Cynodon* species studied form a polyploid series with a basic number of 10, in which *Cynodon transvaalensis* is the diploid form,

Cynodon Magennisii the triploid, and *Cynodon dactylon* and *Cynodon dactylon* var. *densus* both tetraploid forms.

It is difficult to ascertain the cytological relationship of *Cynodon Bradleyi* to the other species. It is possible that this grass may be an aneuploid; as a result of non-disjunction or unequal distribution of the chromosomes, gametes with one chromosome less than the usual number may arise, and the union of two such $n-1$ gametes would result in a form with one pair less than the usual somatic complement. According to Sharp (1926) such aneuploid forms differ more from the normal diploid than do polyploid forms. Church (1929) came across several such examples in his study of grass genera. He found it convenient to use the term "dysploids" for these "irregular deviations from the fundamental haploid base in a polyploid series". He also discovered that many of these "dysploids" had an obvious hybrid origin, and came to the conclusion that hybridity and dysploidy were frequently correlated. The fact that *Cynodon Bradleyi* is completely sterile suggests that this grass may also be of hybrid origin. Such an origin might certainly account for its unusual chromosome number.

Alternatively, it may be suggested that the *Cynodon* genus has two basic numbers of 9 and 10, and that the genus has therefore developed along two separate lines. On this assumption, it would be difficult to explain why *Cynodon Bradleyi*, the diploid form in the 9-series, is completely sterile. If two groups are present in the genus, one would expect to find very distinct morphological and anatomical differences between *Cynodon Bradleyi* on the one hand, and the other species on the other. Differences do exist; *Cynodon Bradleyi* is distinct from the other species as regards the leaf vernation and the absence of underground rhizomes. However, these differences would appear too slight to provide the evidence in support of such an assumption.

A careful study of the external morphology and leaf anatomy of *Cynodon Magennisii* suggests that this grass is intermediate between *Cynodon dactylon* and *Cynodon transvaalensis*. In his description of the origin of this grass (Mathews, 1935), Mr. W. B. Magennis definitely considers it to be a hybrid between "Florida" (*Cynodon transvaalensis*) and "Kweek" (*Cynodon dactylon*), and the chromosome number supports his contention. Historical, morphological and cytological data, therefore, suggest that this triploid is not an auto-polyploid but a definite allo-polyploid arising from a cross between a diploid and a tetraploid. The fact that *Cynodon Magennisii* does not set seed supports the observation made by many workers that triploids are nearly always sexually sterile, since they cannot undergo regular reduction owing to the odd number of chromosomes.

That *Cynodon dactylon* is a tetraploid serves to explain many of the characteristics of this species. As is often typical of tetraploid forms this grass is larger in size, more vigorous in growth, and altogether hardier than the diploid form. *Cynodon dactylon* has a world-wide distribution and flourishes under a variety of conditions, whereas other *Cynodon* species are of limited distribution and are often restricted to one particular habitat. Many systematists have remarked on the width of variation which occurs within this one species. According to Crane (1940) such variation is obviously correlated with the tetraploid nature of the plant.

It is interesting to note that *C. dactylon* is highly fertile, whereas *C. transvaalensis* rarely produces mature seed. Darlington (1940) observes that a polyploid which has arisen from a non-hybrid diploid is less fertile than the diploid parent, whereas doubling of the chromosomes in a more or less sterile hybrid results in a polyploid which is fairly fertile and, in proportion to its fertility, true-breeding. It would appear, therefore, that the origin of a fertile tetraploid has a sound genetic basis, and that *C. dactylon* possibly arose from the doubling of chromosomes in a fairly sterile diploid ancestor.

C. dactylon var. *densus* (Hall's Selection) resembles *C. dactylon* in many respects; both have the same chromosome number, their leaf anatomy is almost identical, and as regards most features of external morphology they are closely similar. But whereas *C. dactylon* is a vigorous grass which may reach a height of 40 cm. and is fairly fertile, *C. dactylon* var. *densus* is a slow-growing, prostrate, tufted grass which rarely sets seed. The available data, therefore, suggest that "Hall's Selection" is merely a variety of *C. dactylon*.

Stent (1927) considers both *C. Bradleyi* and *C. transvaalensis* to be natural hybrids between *C. dactylon* and *C. hirsutus*. This does not seem probable in the light of a numerical comparison of the chromosomes of the three first-mentioned species. However, the chromosome number of *C. hirsutus* must be determined before any statement can be made. Certainly the fact that *C. transvaalensis* rarely sets seed and that *C. Bradleyi* is completely sterile does suggest that these two species might have a hybrid origin.

COMPARISON OF THE CHROMOSOME NUMBERS WITH THOSE RECORDED IN THE LITERATURE.

Hunter's chromosome number of 30 for *C. dactylon* supports the writer's assumption that 10 is the basic number of the *Cynodon* genus. But, whereas the writer found *C. dactylon* to be a tetraploid, Hunter finds it to be a triploid.

The numbers obtained by both Janaki Ammal and Avdulow for *Cynodon* species are at variance with those determined by the writer. These two workers maintain that the basic number of the *Cynodon* genus is 9, since they both find 36 chromosomes in *C. dactylon* and Janaki Ammal records a diploid count of 18 for *C. diploidum*.

To assume that the *Cynodon* genus has two basic numbers of 9 and 10 corresponding to two separate lines of development would appear to solve this problem of conflicting chromosome numbers. However, the reasons why the writer considers *C. Bradleyi* to be an aneuploid species derived from an ancestor with a basic number of 10 rather than a diploid species with a basic number of 9, have already been given. Also, *C. dactylon* according to Janaki Ammal and Avdulow should fall into the 9-series, while according to the writer it should be included in the 10-series. From a study of the literature and of this present work, it is clear that *Cynodon dactylon* is reported to have three different chromosome numbers—30, 36 and 40. Unfortunately, not one of the workers mentioned describes the morphology of the grass studied, so that it is impossible to make any comparison. It is therefore probable that these three numbers refer not to one species, but to three different species of *Cynodon*. It appears therefore that the problem concerning the basic number of the *Cynodon* genus cannot be solved until the chromosome complements of all the known species within the genus have been critically examined. The writer is convinced that such a cytological examination must be accompanied by a careful morphological description of each grass studied.

CONCLUSIONS :

The problem of the taxonomic relationships of the *Cynodon* species selected was elucidated by a study of their external morphology, leaf anatomy, and chromosome number. The results of the cytological and morphological research showed a remarkable uniformity, and it is certain that the number of chromosomes was a useful additional character in the determination of the position of these species whose relationships would otherwise have been uncertain.

A detailed examination of the external morphology showed that the species do not differ greatly. The only reliable and constant characters are the veneration of the leaves, the presence or absence of rhizomes, the nature of the produced rachilla, and the length of the glumes in relation to that of the spikelet.

From a critical study of the leaf anatomy it was concluded that the only reliable diagnostic features are the nature of the abaxial epidermis and the number of first order bundles present. A diagnostic key to the

species was based on characters of external morphology in conjunction with anatomical characters.

The chromosome numbers indicated that the basic number of the genus is 10 and that the species form a polyploid series in which *Cynodon transvaalensis* is the diploid form, *Cynodon Magennisii* the triploid, and *Cynodon dactylon* and *Cynodon dactylon* var. *densus* both tetraploid forms. *Cynodon Bradleyi* with a somatic number of 18 was assumed to be an aneuploid species.

A careful study of all the available data led to the following conclusions as regards the systematic relationship of the *Cynodon* species studied :

- (1) *Cynodon dactylon* Pers., *Cynodon transvaalensis* Burtt Davy and *C. Bradleyi* Stent were found to be well-defined species.
- (2) *Cynodon Magennisii* sp. nov. was found to be a hybrid between *Cynodon dactylon* and *Cynodon transvaalensis*.
The writer conferred specific rank on this hybrid because it remains true to type as it reproduces only by vegetative means.
- (3) The grass known commonly as "Hall's Selection" differed from *Cynodon dactylon* only as regards size and growth form. The writer therefore considered this grass to be a variety of *Cynodon dactylon* and referred to it as *Cynodon dactylon* Pers. var. *densus* var. nov.

The above paper is a summary of a thesis presented for the degree of M.Sc. in Botany at the University of the Witwatersrand.

The writer is continuing with this work and is at present studying the remaining South African *Cynodon* species, namely, *Cynodon incompletus* Nees, *Cynodon hirsutus* Stent and *Cynodon plectostachyum* Pilg.

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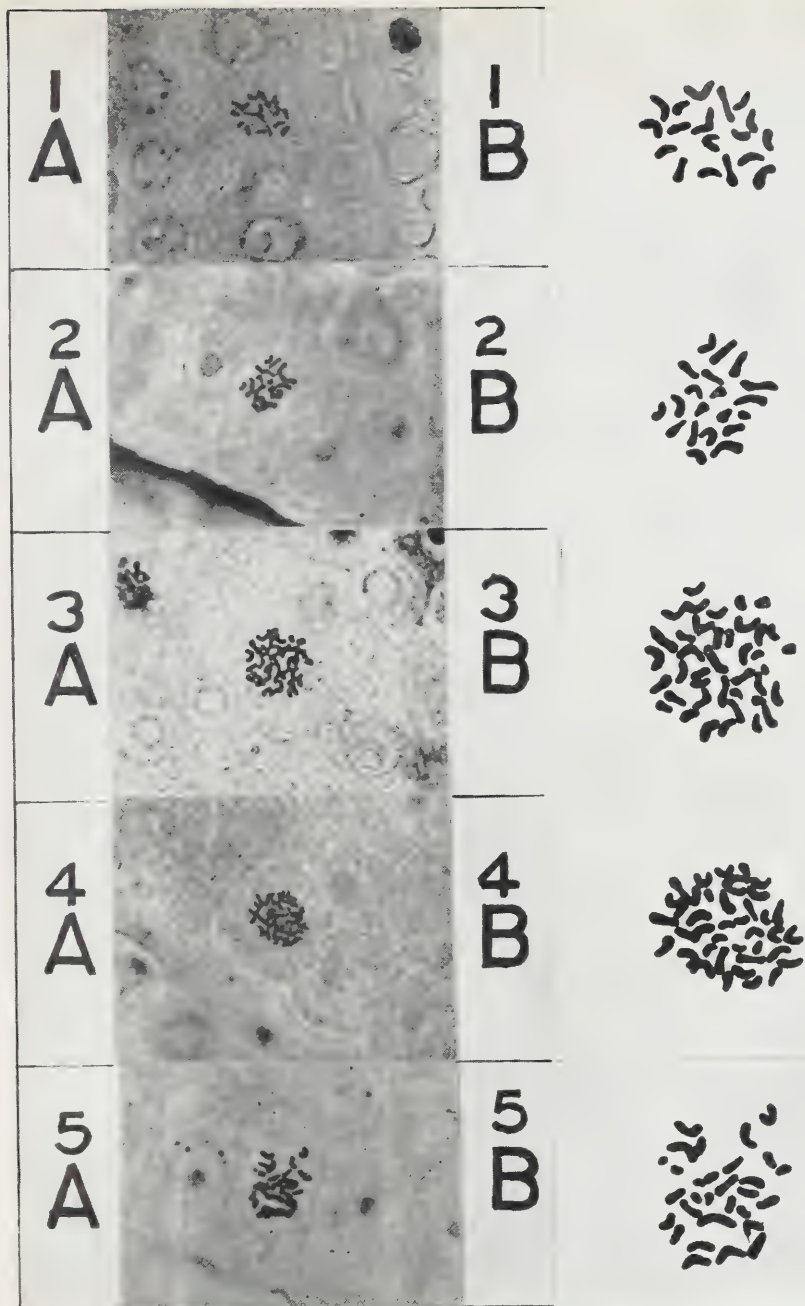


PLATE XVII.

SOMATIC CHROMOSOMES OF CYNODON SPECIES.

1. *C. Bradleyi* Stent.

2. *C. transvaalensis* Burt Davy.

3. *C. dactylon* Pers.

4. *C. dactylon* Pers var. *densus* var. nov.

5. *C. Magennisii* sp. nov.

1A-5A: Photomicrographs of Metaphase Plates, 1150 $\times$.

1B-5B: Camera Lucida Drawings of Metaphase Plates 3,000 $\times$.